

Development of selectively lysine decarboxylase broth using spectrophotometric assay for *Salmonella* screening

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Abstract

This study was to demonstrate the application of spectrophotometry for rapid and presumptive differentiation of *Salmonella* from other Enterobacteriaceae based on lysine decarboxylase (LDC) activity. The pH indicator system and cocktails of *Salmonella* selective inhibitors were optimized to derive a new selective broth (patent pending no. 1301006577) for *Salmonella* screening. Phenol red (PR) was the most suitable pH indicator for the lysine decarboxylase broth and then derived a new medium, developed lysine decarboxylase broth with phenol red (dLDBPR). This enrichment medium was able to produce the highest absorbance to differentiate LDC-positive from -negative samples at 587 nm wavelength and proven to be rapid and useful approach to identify salmonellae and Enterobacteriaceae from other competitors. The variation of bacterial growth and broth pH generated from the glucose fermentation and LDC activity resulted in broth color changes. The most efficient broth was dLDBPR with RVS inhibitors (dLRVS) enabling fast positive-color shift within 8-10 h and sustained cell viability in all LDC-positive *Salmonella* samples. However, dLRVS treatment contained some false LDC-positive results in some samples with non-*Salmonella* competitors (*Escherichia coli* and *Klebsiella pneumoniae*). Further optimization of selective inhibitors towards *Salmonella* detection is in progress. The assay was proven to be rapid and useful approach to distinguish salmonellae and Enterobacteriaceae from other competitors.

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Introduction

Consumption of *Salmonella*-contaminated food can potentially cause diarrheal illness, and salmonellosis often produces sporadic food-borne outbreaks worldwide (Torlak *et al.*, 2012). Many types of food and beverage can carry *Salmonella* linked to foodborne outbreaks, although most common sources are food products from animal origin (Forshell and Wierup, 2006; Alakomi and Saarela, 2009). Of several major foodborne pathogens, *Salmonella* imposes the most critical impact on public health and economic cost (Hoffmann and Anekwe, 2013; CDC, 2014). In 2011, a report of Center for Disease Control and Prevention (CDC) indicated that non-typhoid *Salmonella* inflicted approximately 1 million cases from the combination of illnesses, hospitalization and death. Hence, they ranked the costliest in expense for medical care, lost time from work, and losses due to premature death.

The incidences of *Salmonella* outbreaks have emphasized the importance of food safety in food manufacturing. Many countries have issues rigorous

regulations and inspection for domestic and export food products to meet the highest food safety. There is an emerging interest in industrial food manufacturers and research community to utilize more advanced detection methods for the production routine. Therefore, the ideal detection technique must enable accurate and rapid identification of *Salmonella* in food products prior to distribution to consumers.

Most of the universal standards for the detection of *Salmonella* spp. from food samples consist of four main steps, beginning with a non-selective pre-enrichment, followed by a selective enrichment step, then isolation on selective agar media and finally a preliminary biochemical and serological confirmation. This conventional culture method is very time consuming, labor-intensive, and high analytical cost (Walker *et al.*, 2001). Altogether it is an inefficient detection for industrial routine and always subject to constant revision by the standardization committees (Schönenbrücher *et al.*, 2008). Current ISO method for the detection of *Salmonella* in foods requires minimum 3 days to obtain presumptive positive or negative results (ISO, 2002). The proposed concept

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of industrial food microbiology is to determine the negative result as early as possible. The rapid results are necessary to take timely precautions against possible microbiological food safety risks. The reduction of analytical time can be achieved by utilizing specific biochemical properties enabling differentiation of *Salmonella* from other competing microbes.

There are two biochemical reactions (lysine decarboxylation and hydrogen sulfide production) that are practical to incorporate into the selective enrichment broths. All typhoid and non-typhoid *Salmonella* serotypes (except paratyphoid *Salmonella* Paratyphi A) possess lysine decarboxylase and rapidly decarboxylate lysine. Most non-salmonellae within Enterobacteriaceae do not have lysine decarboxylase (Shelef *et al.*, 1998; Morita *et al.*, 2006). The use of hydrogen sulfide as a substrate for ferric sulfide precipitation can be troublesome when applying spectrophotometry. The increase of alkalinity as a result of lysine decarboxylation is a unique biochemical reaction of *Salmonella* spp. in liquid suspension. This biochemical test was already well-established in the biochemical confirmation. To our knowledge, it has never been applied to the selective enrichment in suspended cell culture. Our proposed technique to enhance the selective enrichment was to allow lysine decarboxylation to take place in the enrichment step and utilize pH indicators (i.e., bromocresol purple, bromothymol blue, and phenol red) to detect the pH changes. The objective was to demonstrate the use of lysine decarboxylase broth and the spectrophotometric detection of broth color to identify samples without *Salmonella* contamination. The negative result from this technique is conclusive that the food samples do not have *Salmonella* contamination. The samples with positive results require further analysis to identify whether the contamination is from *Salmonella* or other DC containing strains.

Materials and Methods

Culture preparation

All bacteria were obtained from the Department of Medical Sciences Thailand (DMST, Bangkok, Thailand) and Thailand Institute of Scientific and Technological Research (TISTR, Bangkok, Thailand). *Salmonella* were 4 non-typhoid serovars (*Salmonella* Anatum, DMST 19600; *Salmonella* Choleraesuis, DMST 8014; *Salmonella* Enteritidis, DMST 15673; *Salmonella* Gallinarum, DMST 15968) and typhoid (*Salmonella* Typhi, DMST 22842) and paratyphoid (*Salmonella* Paratyphi A, DMST 15673). The Gram-

negative competitive bacteria included *Escherichia coli*, DMST 4609; *Klebsiella pneumoniae*, DMST 8216 as representatives of LDC-positive bacteria. While *Proteus vulgaris*, DMST 557 was LDC-negative Gram-negative competitor. Few Gram-positive competitive bacteria, *Enterococcus faecalis*, DMST 4736 and *Staphylococcus aureus*, TISTR 808 were also tested because they were sensitive to most inhibitors (Arroyo and Arroyo, 1995).

All pure cultures were sub-cultured on tryptic soy agar (TSA, Lab M, UK) and one loopful of each strain was transferred into 10 ml of tryptic soy broth (TSB, Lab M, UK) in a 11-ml glass tube and incubated under a static condition at 37°C for 24 h. The 10-fold serial dilutions were then done in 0.1% w/v peptone water (PW, Difco Laboratories, Sparks, MD) to the desired concentration.

Testing media and pH indicators

Developed lysine decarboxylase broth (dLDB) adapted from the original formula proposed by Falkow (1958) with soytone (4.5 g/l; USbiological, USA), D-glucose (1 g/l; Merck, Germany) and L-lysine (5 g/l; USbiological, Salem, MA). The dLDB base was added with a pH indicator bromocresol purple (0.02 g/l, BP; Fisher Scientific, Fair Lawn, NJ), or bromothymol blue (0.065 g/l, BB; Acros Organics, Fair Lawn, NJ) or phenol red (0.08 g/l, PR; Acros organics, NJ) for spectrophotometric evaluation of the optimum pH indicator under the artificial and realistic conditions. For artificial condition, pH of the media with each pH indicator was adjusted into 3 ranges: neutral (pH 7), basic (pH 8, 8.5, 9) and acidic (pH 5, 5.5, 6) by NaOH (Carlo Erba, France) 1 N and HCl (QRĕC®, Malaysia) 1 N using the pH meter (Mettler Toledo, S220 SevenCompact™ pH/Ion Meters) with a pH electrode (Mettler Toledo, uPlace™). Visible wavelength (330-800 nm) absorption spectra of the dLDBBP, dLDBBB, and dLDBPR at pH 5.0 to 9.0 (Figure 2) were obtained by the spectrophotometer (SP-880, Metertech, Taiwan). The absorbance difference (peak absorption of the media at each pH – peak absorption of the media at pH 7) was determined (Figure 2). The wavelength showing highest absorbance difference was used for spectrophotometric detection of salmonellae.

Phenol red was chosen as the optimum pH indicator. The inhibitors were derived from the 3 *Salmonella* standard selective broths, Müller-Kauffmann tetrathionate novobiocin broth (MKTTn), selenite cystine broth (SC) and Rappaport-Vassiliadis soy broth (RVS). Selective inhibitors from MKTTn were sodium thiosulfate (47.8 g/l, Acros Organics, Fair Lawn, NJ), Oxgall (4.78 g/l, USbiological,

Salem, MA), brilliant green (0.0096 g/l, Fisher Scientific, Fair Lawn, NJ), novobiocin 0.8% w/v solution (5 ml/l, Bio-Rad, Marnes-la-Coquette, France) and iodine solution (20 ml/l, Carlo Erba Reagents, Italy). SC inhibitors were sodium selenite (4 g/l, MP Biomedicals, Solon, OH) and L-cystine (0.01 g/l, Fisher Scientific, Fair Lawn, NJ). RVS selective agents were malachite green (0.036 g/l, Acros Organics, Fair Lawn, NJ) and magnesium chloride anhydrous (28.6 g/l, Carlo Erba, France). The inhibitors in standard selective agars, sodium deoxycholate (2.5 g/l, TSB, Difco Laboratories, Sparks, MD), bile salts (9 g/l, Difco Laboratories, Sparks, MD) and brilliant green (0.025 g/l), were also studied. In addition, the selective agents, in a newer selective medium, KIMAN, developed by Blivet *et al.* (1997) which were composed of potassium iodide (40 g/l, Carlo Erba, France), malachite green (10 mg/l) and novobiocin (20 mg/l) were tested. All selective broths were adjusted to the initial pH of 7.0 ± 0.2 (red) and then sterilized before using.

Lysine decarboxylation and effect of selective agents on absorbance changes and viable cell counts of Salmonella and their competitors

Pure cultures salmonellae and non-salmonellae bacteria (at approximately $7 \log$ CFU/ml) were inoculated into a sterile transparent cuvette (3 ml total volume), containing 1.8 ml of each medium broth (dLDBPR with and without standard inhibitors). The reactors were then incubated at $37 \pm 1^\circ\text{C}$ for 24 h before spectrophotometric measurement of absorbance at 587 nm. Viable cell counts were done at different time during the 24 h incubation.

Viable cell enumeration was done for each strain using the modified drop plate technique (MDPT) (Khueankhancharoen and Thipayarat, 2011). A $10 \mu\text{l}$ aliquot of sample from each incubated reactor was dropped onto $500 \mu\text{l}$ of tryptic soy agar (TSA, Difco) in a 96-well plate format. The microwell plate was incubated at $37 \pm 1^\circ\text{C}$ for 10-12 h. Colonies of viable cells were counted under a digital microscope.

Results and Discussion

Optimization of spectrophotometric technique for Salmonella detection in lysine decarboxylase broth

The conventional lysine decarboxylase test requires the inoculation of one loopful of cell inoculum into 5 ml of lysine decarboxylase broth (LDB) and 2-day cultivation to determine lysine decarboxylation. The broth color begins with pale violet at neutral pH, develops to yellow under acidic pH from sugar fermentation and successively

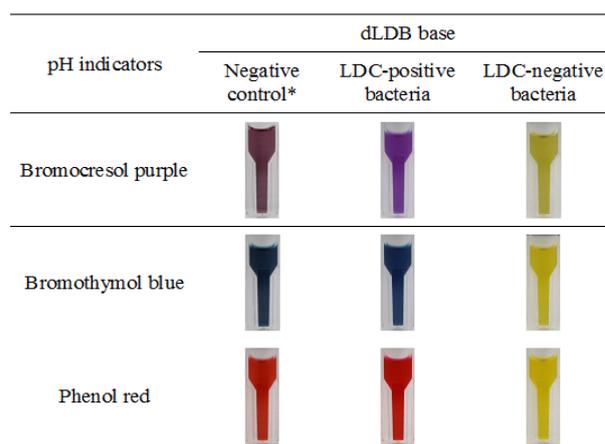


Figure 1. Photographs of cuvettes containing dLDB with 3 different pH indicators, cultured with LDC-positive and -negative bacteria at 37°C for 24 h

*No inoculation

shows purple under basic pH if there is the presence of lysine decarboxylase enzyme (Figure 1). This *Salmonella*'s biochemical confirmation step serves to exhibit the metabolic uniqueness and confirm the presence of *Salmonella* in tested samples (Cowan and Steel, 1965; Akatova *et al.*, 1968). The spectral scan of the bromocresol purple in LDB from pH 5 to 9 was shown in Figure 2a. The differences of the absorbance reading from the control broth indicated that there were a few optimal wavelengths at 430 and 590 nm to differentiate the positive- and negative-samples. In this bromocresol purple system, the negative samples can be identified rather easily due to large differences of absorbance reading between the yellow and pale violet broths. However, the positive results were not straightforward since the pale violet and purple broth had similar absorbance readings and small differences of optical properties.

The use of bromocresol purple in the dLDB as the pH indicator, designated as dLDBBP, showed poor visual and spectrophotometric distinction. There were other literatures reporting the use of appropriate pH indicator system to create high throughput assays to measure enzymatic activities. Yao *et al.* (1998) developed an assay for halohydrin activity by relying on the absorbance change of pH indicators (i.e., brilliant yellow and bromothymol blue). For LDB, two additional pH indicator systems (e.g., bromothymol blue and phenol red) were investigated (Figure 2b and c).

Bromothymol blue provided a slight improvement of bromocresol purple indicator. At the neutral pH, the LDB with bromothymol blue (dLDBBB) appeared as blue-green color. After dextrose fermentation, the acidic broth of dLDBBB showed yellowish color as in the dLDBBP experiment. But when the lysine decarboxylation activated, the high pH broth as the

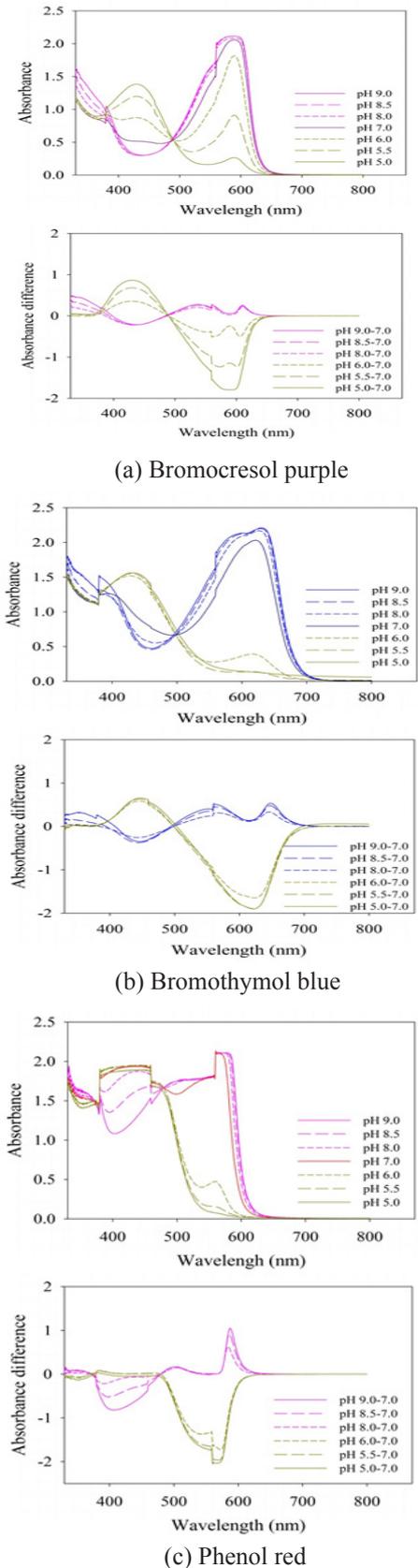


Figure 2. Absorption spectra and absorbance difference spectra of dLDB base with 3 pH indicators; bromocresol purple (a), bromothymol blue (b) and phenol red (c) using a uv-visible spectrophotometer. The broth without inoculum was adjusted to different pH simulating the change in color due to LDC activity.

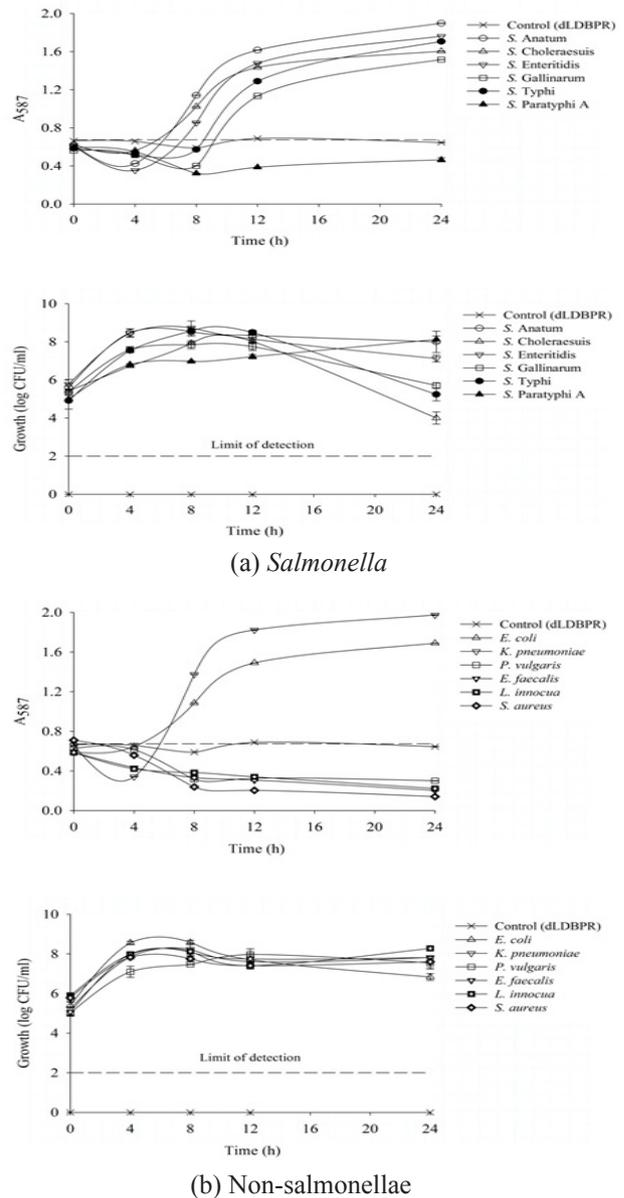


Figure 3. Time course of absorbance (reflecting LDC activity) and cell growth evaluating of (a) *Salmonella* and (b) some non-salmonellae including Gram-negative bacteria in Enterobacteriaceae and Gram-positive bacteria 6-7 log CFU/ml in dLDBPR

result of LDC activity turned the broth color blue. The absorbance readings of the high alkalinity and the control broths in dLDBBB were more distinct and separated apart than those in dLDBBP (Figure 2a and b). Also the absorbance differences of the alkaline broths from the control and acidic treatments were much larger, representing good identification of positive and negative results especially at 430 and 650 nm (Figure 2b). The maximal difference in the absorbance readings between the two pH values was desirable to observe the activity of lysine decarboxylase.

The use of phenol red, on the other hand, generated much improved absorbance differences of

Table 1. The LDC activity and viable cell count of *Salmonella* and non-*Salmonella* strains in dLDBPR with different inhibitor cocktails from the existing *Salmonella* enrichment broths

Test strains	Inhibitors in dLDBPR							
	Control*	MKTn ¹	SC ²	RVS ³	XLD ⁴	HEA ⁵	BGA ⁶	KIMAN ⁷
Viable cell concentration after incubation for 24 h (log CFU/ml)								
<i>Salmonella</i>								
Typical								
<i>S. Anatum</i>	8.00±0.56	4.29±0.23	8.24±0.41	8.42±0.20	3.68±0.24	3.42±0.16	8.09±0.12	6.76±0.20
<i>S. Choleraesuis</i>	4.00±0.32	0.00±0.00	2.35±0.49	7.73±0.32	8.15±0.21	5.29±0.02	6.34±0.62	3.51±0.27
<i>S. Enteritidis</i>	7.13±0.18	0.00±0.00	8.68±0.08	8.37±0.13	6.96±0.26	0.00±0.00	7.96±0.01	7.63±0.46
<i>S. Gallinarum</i>	5.71±0.15	3.30±0.43	6.84±0.09	7.79±0.23	3.94±0.41	5.03±0.54	7.90±0.43	7.71±0.01
Atypical								
<i>S. Typhi</i>	5.24±0.34	2.95±0.07	4.04±0.15	7.89±0.41	6.95±0.07	8.19±0.27	4.18±0.26	3.37±0.52
<i>S. Paratyphi A</i> (LDC-negative)	8.13±0.18	2.00±0.00	2.15±0.21	7.96±0.17	7.87±0.29	7.87±0.38	6.84±0.09	5.40±0.14
Non-<i>Salmonella</i>								
LDC-positive								
<i>E. coli</i>	7.14±0.17	0.00±0.00	7.37±0.10	8.68±0.10	8.94±0.06	0.00±0.00	2.00±0.00	4.10±0.28
<i>K. pneumoniae</i>	7.82±0.04	0.00±0.00	0.00±0.00	7.99±0.08	5.12±0.19	0.00±0.00	3.68±0.24	3.62±0.48
LDC-negative								
<i>P. vulgaris</i>	7.55±0.32	0.00±0.00	4.14±0.20	7.95±0.19	7.84±0.16	6.95±0.07	3.50±0.71	0.00±0.00
<i>E. faecalis</i>	7.53±0.27	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>L. innocua</i>	8.28±0.09	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>S. aureus</i>	7.60±0.30	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
LDC activity^a after incubation for 24 h								
<i>Salmonella</i>								
Typical								
<i>S. Anatum</i>	+	-	+	+	+	+	+	+
<i>S. Choleraesuis</i>	+	-	-	+	+	+	+	-
<i>S. Enteritidis</i>	+	-	+	+	+	+	+	+
<i>S. Gallinarum</i>	+	-	-	+	+	+	+	-
Atypical								
<i>S. Typhi</i>	+	-	-	+	-	-	-	-
<i>S. Paratyphi A</i> (LDC-negative)	-	-	-	-	-	-	-	-
Non-<i>Salmonella</i>								
LDC-positive								
<i>E. coli</i>	+	-	-	+	+	+	-	-
<i>K. pneumoniae</i>	+	-	-	+	+	+	-	-
LDC-negative								
<i>P. vulgaris</i>	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	-	-	-	-	-	-	-	-
<i>L. innocua</i>	-	-	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-

*Control, dLDBPR without inhibitors.

^aBased on absorbance reading at 587 nm.

the control and alkaline broths (Figure 2c). Unlike the previous two indicators, the control broth color was red initially, turned yellow due to the acidic condition and developed pink color to indicate lysine decarboxylation. The absorbance difference between the red and the pink in dLDBPR was much larger than the other two systems (i.e., dLDBBB and dLDBBP). The optimal wavelength for absorbance readings was at 587 nm. Tang *et al.* (2010) successfully demonstrated the use of phenol red to measure the activity of halohydrin dehalogenases (HheC). The pH changes due to the release of protons from the enzyme catalyzed reactions were able to be successfully monitored via the absorbance reading at 560 nm and visual color change.

Spectrophotometry and viable cell counts of the dLDBPR inoculated with Salmonella and their competitors.

The changes in the absorbance readings in response to acid fermentation (yellow broth), and amine production (reversal to pink), were monitored

over 24 h (Figure 3). Of 6 *Salmonella* strains tested, 1 serovar (*S. Paratyphi A*) was LDC-negative; therefore, it did not develop pink broth and the absorbance reading remained below 0.6-0.7 reflecting yellowish shades (Figure 3a). The remaining of the serovars apparently produced pink broth and raised the absorbance to higher than 1 similar to A_{587} of the simulated dLDBPR in Figure 2c.

As opposed to other LDC-positive strains, *S. Paratyphi A* showed slow but steady growth without cell reduction toward the end of incubation. Figure 3a indicated that *S. Paratyphi A* had an exceptional acid resistance and still multiplied in the acidic dLDBPR. Burin *et al.* (2014) demonstrated the Influence of lactic acid and acetic acid on *Salmonella* spp. growth and expression of acid tolerance-related genes. Under acid stress, some *Salmonella*, like *S. Paratyphi A*, showed a complex tolerance mechanism of survival that involves multiple protein expression, which protects the bacterial cell against damage caused by acid stress (Lange *et al.*, 1995; Paesold and Krause, 1999; Hengge-Aronis, 2002; Lues and Theron, 2011).

The growth profiles of all *Salmonella* serovars showed the initial increase of CFU count during sugar fermentation (Figure 3a). The serovars with LDC enzymes generated higher CFU counts than that without LDC. Viala *et al.* (2011) reported the sensing and adaptation abilities of *Salmonella* to low pH condition. *Salmonella enterica* serovar Typhimurium, for example, tolerated the harsh acid stress of the stomach lining and the inducible lysine decarboxylase promoted its capacity to survive at pH 2.3 and grow at pH 4.5 (Bearson *et al.*, 1998; Audia *et al.*, 2001). It was hypothesized that systems involving an antiporter and an associated amino acid decarboxylase was responsible for the protection of *S. Typhimurium* from an acid shock. *S. Typhimurium* actually possesses three inducible amino acid decarboxylases where decarboxylation of lysine leads to the production of cadaverine that increased the pH of dLDBPR. The LDC-positive strains showed negative growth when the pH of dLDBPR increased. Park *et al.* (2007) also reported a decreased growth rate of *Salmonella* in broth culture at pH 9 in egg white system (Kang *et al.*, 2006).

E. coli and *K. pneumoniae* possess LDC enzymes, they showed similar absorbance and viable cell count profiles to LDC-positive *Salmonella* (Figure 3b). LDC-negative strains such as *P. vulgaris*, *E. faecalis*, *S. aureus* and *S. Paratyphi A* showed similar A587. It is apparent in Figure 3 that the detection LDC activity can be achieved within 8-10 h in dLDBPR for both *Salmonella* and non-*Salmonella* strains. The spectrophotometric technique at 587 nm with phenol red as the pH indicator improved the detectability and sensitivity of the LDC test. Once the absorbance change can be observed numerically, an automated detection of amino acid decarboxylation for *Salmonella* and non-*Salmonella* can be realized (Shelef *et al.*, 1998). Integrated with other phenotypic reactions, decarboxylation can be very useful to automatically differentiate salmonellae from other Enterobacteriaceae.

Effect of selective agents on cell growth and absorbance due to lysine decarboxylase

Practically, proper selective inhibitors must be incorporated to increase selectivity towards *Salmonella* species. The most desirable inhibitory cocktail for dLDBPR should facilitate the growth of *Salmonella* spp. and promote the chromatic change of dLDBPR as a result of LDC activity. Table 1 summarized the LDC activity and viable cell count of *Salmonella* and non-*Salmonella* in dLDBPR with different inhibitor cocktails from the existing *Salmonella* enrichment substrates (ISO, 2002; FDA,

2014). The control treatment without any inhibitor showed no selective preference towards any Gram-positive or -negative microorganisms whereas all added selective agents inhibited all Gram-positive competitors (Table 1). The selective agent used in the standard RVS promoted the growths of all tested *Salmonella* and even surpassed the growth of those cultivated in the control dLDBPR without the inhibitor.

It was hypothesized that high concentration of magnesium chloride in RVS helped regulate the tonicity of the enrichment medium at low pH (Taskila *et al.*, 2012). Together with malachite green, they provided good inhibitory effect against Gram-positive bacteria while minimally affected the recovery and growth of *Salmonella*. RVS medium in general has been proved to be superior to other selective enrichment substrates (e.g., TT, MKTBG, MKTTn, and SC) in many studies despite the fact that it still permits the growth of other enteric bacteria (Rhodes *et al.*, 1985; Majjala *et al.*, 1992; June *et al.*, 1995; Krascsenicsova *et al.*, 2006; Schöenbrücher *et al.*, 2008). In this study, the selective agents derived from HEA and MKTTn showed poor selectivity for *Salmonella* and entirely inhibited the growth of *S. Enteritidis* and both *S. Enteritidis* and *S. Choleraesuis*, respectively (Table 1). The cell viability and broth color development reflecting the LDC activity in dLDBPR sometimes did not correlate well. *S. Choleraesuis* and *S. Enteritidis* in dLDBPR with MKTTn selective agents had poor cell viability but showed clear pink color development. *S. Typhi* in the dLDBPR with XLD and HEA inhibitors showed high cell growth but no color development.

The combination of dLDBPR and RVS selective inhibitors (dLRVS) produced good correlation between the cell viability and the decarboxylase color development. In industrial application, dLRVS is potentially useful for safety *Salmonella* screening. This broth has a sensitive color indicator and minimal inhibitory effect, consequently no false-negative result. Therefore, the validation of new dLRVS broth in food samples should be further studied.

Conclusion

The use of LDC activity provided a good indication for *Salmonella* screening. Among three pH indicators tested, phenol red produced reliable A587 reading to differentiate the LDC-positive apart from LDC-negative results. The absorbance measurement of dLDBPR corresponded well with visual detection of broth color but provided fast numerical evaluation using a spectrophotometer. RVS inhibitor cocktail in

dLDBPR promoted the growth of all six *Salmonella* serovars and effectively inhibited Gram-positive competitors such as *E. faecalis*, *L. innocua*, and *S. aureus*. The addition of RVS inhibitors did not affect selectivity of dLDBPR toward *Salmonella* but further enhanced the sensitivity of spectrophotometric measurement indirectly. The dLRVS is potentially a new effective indicative broth for rapid precautionary *Salmonella* screening without the serious false-negative results.

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References

- Akatova, N. S., Ershova, E. B. and Lifanova, I.I. 1968. On the identification of atypical cultures of enterobacteria. *Lab Delo* 6: 329-332.
- Alakomi, H. L. and Saarela, M. 2009. *Salmonella* importance and current status of detection and surveillance methods. *Quality Assurance and Safety of Crops and Foods* 1(3): 142-152.
- Arroyo, G. and Arroyo, J. A. 1995. Selective action of inhibitors used in different culture media on the competitive microflora of *Salmonella*. *Journal of Applied Bacteriology* 78(3): 281-289.
- Audia, J. P., Webb, C. C. and Foster, J. W. 2001. Breaking through the acid barrier: An orchestrated response to proton stress by enteric bacteria. *International Journal of Medical Microbiology* 291(2): 97-106.
- Bearson, B. L., Wilson, L. and Foster, J. W. 1998. A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress. *Journal of Bacteriology* 180(9): 2409-2417.
- Blivet, D., Salvat, G., Humbert, F. and Colin, P. 1997. Evaluation of a new enrichment broth for the isolation of *Salmonella* spp. from poultry products. *International Journal of Food Microbiology* 38(2-3): 211-216.
- Burin, R. C. K., Silva Jr, A. and Nero, L. A. 2014. Influence of lactic acid and acetic acid on *Salmonella* spp. growth and expression of acid tolerance-related genes. *Food Research International* 64(0): 726-732.
- Cowan, S.T. and Steel, K.J. 1965. *Manual for the identification of medical science* (3rd Edition ed.): Cambridge University Press.
- Center for Disease Control and Prevention (CDC). (January 2014). *Bad bug book – Aflatoxins*. Retrieved on September 3, 2014 from CDC Website: www.cdc.gov/foodborneburden/2011-foodborne-estimates.html#annual
- Food and Drug Administration (FDA). (January 2014). *Bacteriological Analytical Manual*, Chapter 15 *Salmonella*. Retrieved on September 3, 2014 from FDA Website: <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm>
- June, G. A., Sherrod, P. S., Hammack, T. S., Amaguana, R. M. and Andrews, W. H. 1995. Relative effectiveness of selenite cystine broth, tetrathionate broth, and Rappaport-Vassiliadis medium for the recovery of *Salmonella* from raw flesh and other highly contaminated foods: precollaborative study. *Journal of AOAC International* 78(2): 375-380.
- Khueankhancharoen, J. and Thipayarat, A. 2011. Application of modified drop plate technique (MDPT) and logistic model to optimize non-selective substrates for *Salmonella typhi* resuscitation. *Asian Journal of Food and Agro-Industry* 4(6): 349-358.
- Krascenicsova, K., Kaclikova, E. and Kuchta, T. 2006. Growth of *Salmonella enterica* in model mixed cultures during a two-step enrichment. *New Microbiologica* 29(4): 261-267.
- Falkow, S. 1958. Activity of lysine decarboxylase as an aid in the identification of *Salmonellae* and *Shigellae*. *Technical bulletin of the Registry of Medical Technologists* 28(5): 106-108.
- Forshell, L. P. and Wierup, M. 2006. *Salmonella* contamination: a significant challenge to the global marketing of animal food products. *Revue Scientifique Et Technique-Office International Des Epizooties* 25(2): 541-554.
- Hengge-Aronis, R. 2002. Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. *Microbiology and Molecular Biology Reviews* 66(3): 373-395.
- Hoffmann S. and Anekwe, T.D. 2013. Making sense of recent cost-of-foodborne-illness estimates. *Economic Information Bulletin*. USDA: Economic Research Service.
- International Organization for Standardization (ISO). 2002. ISO 6579. *Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp.* In International Organization for Standardization. Geneva.
- Kang, H., Loui, C., Clavijo, R. I., Riley, L. W. and Lu, S. 2006. Survival characteristics of *Salmonella enterica* serovar Enteritidis in chicken egg albumen. *Epidemiology and Infection* 134(5): 967-976.
- Lange, R., Fischer, D. and Hengge-Aronis, R. 1995. Identification of transcriptional start sites and the role of ppGpp in the expression of rpoS, the structural gene for the sigma S subunit of RNA polymerase in *Escherichia coli*. *Journal of Bacteriology* 177(16): 4676-4680.
- Lues, J. F. and Theron, M. M. 2012. Comparing organic acids and salt derivatives as antimicrobials against

- selected poultry-borne *Listeria monocytogenes* strains in vitro. Foodborne Pathogens and Disease 9(12): 1126-1129.
- Maijala, R., Johansson, T. and Hirn, J. 1992. Growth of *Salmonella* and competing flora in five commercial Rappaport-Vassiliadis (RV)-media. International Journal of Food Microbiology 17(1): 1-8.
- Morita, M., Mori, K., Tominaga, K., Terajima, J., Hirose, K., Watanabe, H. and Izumiya, H. 2006. Characterization of lysine decarboxylase-negative strains of *Salmonella enterica* serovar Enteritidis disseminated in Japan. FEMS Immunology and Medical Microbiology 46(3): 381-385.
- Park, S. Y., Seo, K. Y. and Ha, S. D. 2007. A response surface model based on absorbance data for the growth rates of *Salmonella enterica* serovar Typhimurium as a function of temperature, NaCl, and pH. Journal of Microbiology and Biotechnology 17(4): 644-649.
- Paesold, G. and Krause, M. 1999. Analysis of rpoS mRNA in *Salmonella dublin*: identification of multiple transcripts with growth-phase-dependent variation in transcript stability. Journal of Bacteriology 181(4): 1264-1268.
- Rhodes, P., Quesnel, L. B. and Collard, P. 1985. Growth kinetics of mixed culture in *Salmonella* enrichment media. Journal of Applied Bacteriology 59(3): 231-237.
- Schönenbrücher, V., Mallinson, E. T. and Bulte, M. 2008. A comparison of standard cultural methods for the detection of foodborne *Salmonella* species including three new chromogenic plating media. International Journal of Food Microbiology 123(1-2): 61-66.
- Shelef, L. A., Surtani, A., Kanagapandian K. and Tan W. 1998. Automated detection of amino acid decarboxylation in salmonellae and other Enterobacteriaceae. Food Microbiology 15(2): 199-205.
- Tang, L., Li, Y. and Wang, X. 2010. A high-throughput colorimetric assay for screening halohydrin dehalogenase saturation mutagenesis libraries. Journal of Biotechnology 147(3-4): 164-168.
- Taskila, S., Tuomola, M. and Ojamo, H. 2012. Enrichment cultivation in detection of food-borne *Salmonella*. Food Control 26(2): 369-377.
- Torlak, E., Akan, I.M. and Inal, M. 2012. Evaluation of RapidChek Select for the screening of *Salmonella* in meat and meat products. Journal of Microbiological Methods 90(3): 217-219.
- Viala, J. P., Meresse, S., Pocachard, B., Guilhon, A. A., Aussel, L. and Barras, F. 2011. Sensing and adaptation to low pH mediated by inducible amino acid decarboxylases in *Salmonella*. Plos One 6(7): e22397.
- Walker, R. L., Kinde, H., Anderson, R. J. and Brown, A. E. 2001. Comparison of VIDAS enzyme-linked fluorescent immunoassay using Moore swab sampling and conventional culture method for *Salmonella* detection in bulk tank milk and in-line milk filters in California dairies. International Journal of Food Microbiology 67(1-2): 123-129.
- Yao, Y., Wang, M. H., Zhao, K. Y., and Wang, C. C. 1998. Assay for enzyme activity by following the absorbance change of pH-indicators. Journal of Biochemical and Biophysical Methods 36(2-3): 119-130.